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Podocyturia parallels proximal tubule dysfunction in type 2 diabetes mellitus patients independently of albuminuria and renal function decline: A cross-sectional study

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ABSTRACT

Aims: Detection of podocytes in the urine of patients with type 2 diabetes may indicate severe injury to the podocytes. In the course of type 2 diabetes the proximal tubule is involved in urinary albumin processing. We studied the significance of podocyturia in relation with proximal tubule dysfunction in type 2 diabetes.
Methods: A total of 86 patients with type 2 diabetes (34-normoalbuminuria; 30-microalbuminuria; 22-macroalbuminuria) and 28 healthy subjects were enrolled in the study and assessed concerning urinary podocytes, podocyte-associated molecules, and biomarkers of proximal tubule dysfunction. Urinary podocytes were examined in cell cultures by utilizing monoclonal antibodies against podocalyxin and synaptopodin.
Results: Podocytes were detected in the urine of 10% of the healthy controls, 24% of the normoalbuminuric, 40% of the microalbuminuric, and 82% of the macroalbuminuric patients. In multivariate logistic regression analysis, urinary podocytes correlated with urinary albumin:creatinine ratio ($p = 0.006$), urinary nephrin/creat ($p = 0.001$), urinary vascular endothelial growth factor/creat ($p = 0.001$), urinary kidney injury molecule-1/creat ($p = 0.003$), cystatin C ($p = 0.001$), urinary advanced glycation end-products ($p = 0.002$), eGFR ($p = 0.001$).
Conclusions: In patients with type 2 diabetes podocyturia parallels proximal tubule dysfunction independently of albuminuria and renal function decline. Advanced glycation end-products may impact the podocytes and the proximal tubule.

1. Introduction

Diabetes mellitus is the leading cause of end-stage renal disease worldwide. The growth in the number of patients with diabetic chronic kidney disease explains why over 40% of patients referred to renal replacement therapies are represented by patients with both type 1 and type 2 diabetes (U. S. Renal Data System, 2013).

The classical concept concerning mechanisms of albuminuria in diabetic nephropathy (DN) relies on defects in the structure of the glomerular filtration barrier (Jefferson, Shankland, & Pichler, 2008; Nakagawa et al., 2011). According to this hypothesis, it is considered that albuminuria is determined by the severity of the glomerular lesions, but this correlation is not strict in preceding the onset of albuminuria, a fact demonstrated on iterated renal biopsies in patients with type 2 diabetes (Fioretto & Mauer, 2007).

The tubular theory concerning albuminuria in the course of diabetes mellitus states that albuminuria is caused primarily by impaired tubular uptake of intact albumin rather than by an increased leakiness of the glomerular filtration barrier (Comper, Haraldsson, & Deen, 2008; Russo et al., 2009). In previous works performed by us in normoalbuminuric patients with type 2 diabetes we demonstrated

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that proximal tubule (PT) dysfunction precedes the occurrence of albuminuria. It is possible that in early DN, sequentially, PT dysfunction may precede glomerular injury (Petrica, Petrica, et al., 2011; Petrica, Vlad, et al., 2011). The glomerulus normally leaks nephrotic levels of albumin, retrieved and processed by the PT (Comper et al., 2008; Russo et al., 2009). The signaling system from the glomerulus to the PT may turn off the pathway of intact albumin in the course of diabetes mellitus. A similar mechanism occurs for many glomerular/podocyte structural perturbations (Russo et al., 2013).

Podocytes are highly specialized epithelial cells which cover the outer aspect of the glomerular basement membrane, playing an important role in the function of the glomerular filtration barrier. Detection of podocytes in the urinary sediment of various glomerular diseases has been shown to indicate severe injury to the podocytes. Urinary podocytes may be a useful marker of disease activity in DN (Nakamura et al., 2000).

Nephrin, a transmembrane protein of the immunoglobulin superfamily, is an important component of the slit diaphragm located between the foot processes of the podocytes. Its alterations lead to the limitation of the size-selectivity of the slit diaphragm (Jefferson et al., 2008).

Increased levels of nephrinuria may be found in type 1 and type 2 diabetes patients with normoalbuminuria, a fact which demonstrates that nephrinuria may precede microalbuminuria (Jim et al., 2012; Ng et al., 2011; Pätäri et al., 2003).

Vascular endothelial growth factor (VEGF) is a pro-angiogenic factor, produced mainly by the podocytes. Urinary excretion of VEGF may increase even in the normoalbuminuria stage, a fact which suggests that urinary VEGF may be used as a sensitive biomarker in the diagnosis of early DN (Kim et al., 2004).

Amongst other causative factors related to PT dysfunction, advanced glycation end-products (AGE) have been involved in the pathogenesis of diabetic tubulopathy (Tang, Leung, & Lai, 2011).

In previous studies we showed that in type 2 diabetes there is an association of PT dysfunction with podocyte damage biomarkers, even in normoalbuminuric patients. This observation suggests a potential role of the PT in urinary nephrin and urinary VEGF processing in early DN, a fact which could be related to AGE intervention (Petrica et al., 2014).

The aim of our study was to evaluate the significance of podocyturia in relation with PT dysfunction in type 2 diabetes. We queried whether this phenomenon could be attributed to AGE, which may intervene in glomerular and PT injury.

2. Subjects, Materials and Methods

2.1. Patients'Enrolment Criteria

A total of 86 consecutive patients with type 2 diabetes (34 patients with normoalbuminuria, 30 patients with microalbuminuria, and 22 patients with macroalbuminuria) attending the Outpatient Department of Diabetes and Metabolic Diseases and 28 healthy control subjects were enrolled in a cross-sectional study. The inclusion criteria were duration of diabetes longer than 5 years, normoalbuminuria [urinary albumin:creatinine ratio (UACR) <30 mg/g], microalbuminuria (UACR 30–300 mg/g), or macroalbuminuria (UACR >300 mg/g). Patients were classified according to levels of UACR measured in the ambulatory clinic, prior to inclusion in the study. All patients were on angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers, other antihypertensive agents (including diuretics), and statins. Also, all patients were treated with oral anti-diabetic agents. There were no significant differences between the therapies across the studied groups.

All patients were assessed concerning: urinary alpha$_1$-microglobulin and urinary kidney injury molecule-1 (KIM-1) as biomarkers of PT dysfunction; urinary nephrin and urinary VEGF, as markers of podocyte damage; plasma and urinary AGE; UACR and serum cystatin C. Serum and urinary biomarkers were determined in specimens frozen at −80 °C and thawed before assay. Urinary biomarkers were assessed in the first morning urine (midstream urine), except for urinary AGE, which were determined in the 24-h sample. All study variables were assessed in triplicate on aliquots from the same first morning urine sample, or from the 24-h urine collection, as appropriate. The ELISA assessments were performed as per protocol indicated by the manufacturer in triplicate assessments for each patient from the same aliquote. The inter- or intra-assay coefficients of variance (CV) were indicated according to the data provided by the manufacturer's brochure. Podocytes were assessed in cell cultures performed from the first morning urine (midstream) in all patients. There were no differences in diuretic usage between groups, and SGLT-2 inhibitors were not utilized.

Chronic kidney disease was defined according to the KDIGO Guideline for the Evaluation and Management of Chronic Kidney Disease (KDIGO, 2013).

2.2. Biomarkers of Podocyte Damage

Nephrin was assessed in the first morning urine (midstream urine) specimen by human NPHN (Nephrin) ELISA kit, Cat. No. E-EL-H1901 Elabscience Biotech Co. Ltd., Wuhan, Hubei Province, China. A human NPHN antibody was utilized. The sensitivity of the assessment showed that the minimum detectable dose of human NPHN is 0.1 ng/ml. The detection range is 0.16–10 ng/ml. The repeatability of the test displayed a CV <10%.

VEGF was assessed in the first morning urine (midstream urine) specimen by a VEGF human ELISA kit for the detection of urinary VEGF, Cat. No. ab100663, Abcam, Cambridge, MA, USA. A human VEGF antibody was utilized and the minimum detectable dose of VEGF was typically less than 10 pg/ml. The intra-assay reproducibility was <10% CV, and the inter-assay reproducibility was <12% CV.

2.3. Biomarkers of PT Dysfunction

Alpha$_1$-microglobulin was evaluated in the first morning urine (midstream urine) specimen with N α1-microglobulin kit (Siemens Healthcare Diagnostics, Marburg, Germany) through particle-enhanced immunonephelometry using the BNProSpec System. The reference interval was 12 mg/l or 0.07–5 mg/g creatinine. The intra-assay precision was 2.9–5.2% CV, while the inter-assay precision was 7.4–13.2% CV.

KIM-1 was assessed in the first morning urine (midstream urine) specimen by KIM-1 ELISA test kit for the detection of KIM-1 in human urine, Cat. No. H-RENA-E-001, Bio Assay Works, Ijamsville, MD, USA. A human KIM-1 antibody was utilized and the detection level was set at urinary KIM-1 < 0.150 ng/ml.

2.4. Albuminuria and Cystatin C

Albuminuria was measured in the first morning urine (midstream urine) specimen through immunonephelometry on the BNProSpec System, with N Antiserum to Human Albumin (Siemens Healthcare Diagnostics, Marburg, Germany). Microalbuminuria was defined by UACR between 30 and 300 mg/g, and normoalbuminuria by UACR < 30 mg/g. The N Antiserum to human albumin was evaluated for the assay of urine on a BN System and yielded a within-run CV of 2.2% and a total CV of 2.6% with a mean of 79 mg/l. The results (ten runs, four determinations per run) were evaluated by analysis of variance, according to the manufacturer's brochure. Urine cultures were negative for bacteriuria in all patients.

Cystatin C was assessed in serum with N latex cystatin C kit (Siemens Healthcare Diagnostics, Marburg, Germany) through particle-enhanced immunonephelometry using the BNProSpec System. The reference interval was calculated nonparametrically and was determined to be 0.53–0.95 mg/l. The intra-assay precision was 2.5%

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